Interocular Differences in Macular Pigment Density

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Interocular differences in the optical density of macular pigment were examined. Foveal and parafoveal sensitivities to lights of 460 and 530 nm were measured by heterochromatic flicker photometry for both eyes of ten subjects. These two wavelengths represent the maximum and minimum absorbance for macular pigment. Taking the difference in log sensitivity to the 460 nm light for the fovea and parafovea, after normalizing with respect to 530 nm, yields a measurement of the optical density of the macular pigment. Consistent interocular differences in macular pigment density were found for only two subjects, and these differences were less than 0.1. Other subjects frequently showed significant interocular differences on a given day but showed no consistent differences over the course of many days. In general, the amount of macular pigment measured for one eye was found to be essentially the same as that for the other eye. When measurements were averaged for the two eyes of each subject, significant differences in macular pigment density among subjects were found. Invest Ophthalmol Vis Sci 33:350-355, 1992

The optical density (OD) of macular pigment has been assessed using various techniques. Methods include imaging fundus reflectometry,1 high-performance liquid chromatography,2-4 microdensitometry,5-7 and microspectrophotometry.8 Psychophysical measurements have also been used to assess the OD of macular pigment.9-20 For many of these measurements, macular pigment OD typically is derived by comparing the spectral sensitivity obtained at the fovea to the spectral sensitivity obtained outside of the fovea. Assuming the same relative receptor population between these two retinal loci, or experimentally controlling for this, one can derive a difference spectrum from the two spectral sensitivity curves. The difference between the peak and tail of this difference spectrum is a measurement of the OD of the macular pigment of the eye.

Rarely, have macular pigment OD measurements been obtained for both eyes. In no case, to our knowledge, has this been done psychophysically. Presumably, this is because the macular pigment, like many components of the visual system, is assumed to be bilaterally yoked. Little work has been done to support this assumption. Only two laboratories have investigated interocular differences in macular pigment, and they reached different conclusions. Using microdensitometry, screening density profiles of macular pigment were obtained in ten squirrel monkeys.6 Although there were considerable individual differences in the ODs between monkeys, there was no difference between the two eyes of the same monkey. Using microdensitometry and high-performance liquid chromatography, these findings were replicated in another group of squirrel monkeys and in macaques.4

In an earlier study, interocular differences in macular pigment also were investigated.21 Based on high-performance liquid chromatography on extirpated samples from human donors, interocular differences were found. A similar method with human donors also showed differences3 (as high as 67%, with an average difference of 29% between the two eyes).

Although these methods have the virtue of being direct, it is possible that postmortem degradation of the retina may mask any true interocular differences by increasing the variability of the measurements. It is also possible that such measurement error could suggest differences spuriously where none actually exist. In addition, these studies were done on excised eyes which allow only one OD value to be obtained per subject. In our study, in vivo, psychophysical measurements of macular pigment were made. This approach has the advantage of eliminating any possible intrusion of postmortem effects. It also allows multiple OD measurements to be obtained so that possible differences in macular pigment density over time, in addition to interocular differences, may be examined.

Addressing the issue of interocular differences might contribute to our knowledge of the transport and deposition of the carotenoids that comprise the macular pigment, of which little is known. It also might account for the occasional reports of interocular differences in color perception.22 In addition, for experiments in which stimuli are presented dichopti-
cally for purposes of comparison, the assumption that the proximal stimuli can be considered to be the same would be tested.

Materials and Methods

Subjects

Four men and six women (age range, 19-42 yr) were tested. They were all white, and all were normal trichromats (as assessed by Ishihara pseudoisochromatic test plates and the Farnsworth D-15 test). Three of the subjects were experienced in psychological tasks and aware of the purpose of the study. The remaining subjects had little experience in psychological tasks and remained experimentally naive. All subjects consented to testing after the nature of the experimental task was explained.

Apparatus

A standard four-channel Maxwellian view optical system was used with a 1000-W xenon arc light source. Two channels were combined to provide a test stimulus, consisting of a standard and a comparison field, to be used for heterochromatic flicker photometry. A third channel provided a background field, and a fourth channel provided the fixation points. The subject's eye position was stabilized with an adjustable dental-impression bite bar and headrest assembly. An auxiliary channel with a calibrated reticle was used to align the subject's pupil. This channel also was used periodically to assure that the stimulus always entered the center of the subject's pupil. In one channel was manipulated with a J-Y Optical Systems (Metuchen, NJ) H-10 monochromator (nominal half bandwidth, 8.0 nm); blocking filters were used appropriately in conjunction with the monochromator. Dichroic Optics (Hudson, MA) interference filters (half bandwidth, 7 nm) were used to render the light monochromatic in the other two channels. A sectored mirror, rotated by a highly regulated motor, was placed at the interface of the two channels comprising the test field. A high-speed, electromechanical shutter (Uniblitz AOX5; Vincent Associates, Rochester, NY) with accompanying timer provided a test-field exposure of 1.5 sec every 3.5 sec. Wedge and neutral-density filters were of the reflection type.

Stimulus

The stimulus consisted of a 1° test field, which was composed alternately of a 25-td, 460-nm standard and a 460-nm or 530-nm comparison field. The standard and comparison fields were superposed and presented out of phase at a temporal rate of at least 15 Hz. This field, which served as the test field, was presented in the center of a 450-nm, 4° background. Two fixation points were used for the parafoveal conditions and were placed on each side of the stimulus and on the same horizontal plane. During parafoveal testing, the subjects fixated these points, which were located 8° to the right or left of the test field, depending on which eye was being measured. The temporal retina of each eye was stimulated. Before the experimental session, the position of the fixation points was checked to ensure that the test field was always in the precise center of the fixation points. The focus of the test field and background also was checked, as was the position of the test field with respect to the background to ensure that it was always perfectly concentric.

Procedure

The method used to assess the OD of the macular pigment was nearly identical to that reported previously. With this method, a difference spectrum is derived from the spectral sensitivity of the fovea and that of the parafovea. Spectral sensitivity is measured using heterochromatic flicker photometry (HFP). For our purposes, it was not necessary to measure spectral sensitivity over the entire visible spectrum. Rather, given that the macular pigment absorption spectrum, assessed by the method described earlier, has been well established, it was sufficient and desirable in our case to limit measurements to the peak of the absorption spectrum (approximately, 460 nm) and to a point where absorption by the macular pigment is negligible (in this case, 530 nm). The advantage of limiting measurements to these two wavelengths was that it allowed interocular comparisons to be made in a single session. This was important for the following reason. It is common to encounter day-to-day variability in measuring the OD of macular pigment. Whatever the source of this variability, it could confound any interocular differences obtained if measurements made in one eye on a given day were compared with measurements made in the other eye on another day. Using the full visible spectrum would require such measurements to be made on separate days because of the increased length of the experimental session. To ensure the validity of our truncated measurements, we used the method described subsequently, except for an extended set of wavelengths, to derive a complete difference spectrum for one subject. This was done in one eye only.

After 10 min of dark adaptation, the subject light adapted for 4 min to a 10-td, 450-nm background light presented to the fovea of one eye. This adaptation period was administered for the purpose of eliminating the contribution of the short-wave sensitive
cones. This was necessary because there is evidence that the population of short-wave cones differs across the two retinal locations we studied.\textsuperscript{23,24} If their contribution were not eliminated, then differences in spectral sensitivity at the short-wave end of the spectrum could be an artifact of the differences in short-wave cone contribution and not simply a result of the absorption by the macular pigment. The contribution of this cone class may be eliminated by such selective adaptation and by using a temporal frequency above the critical flicker frequency for short-wave cones. We used a temporal frequency of at least 15 Hz. Others\textsuperscript{14} have shown that the combination of selective adaptation and an appropriate flicker frequency for the test stimulus is sufficient to suppress the contribution of the short-wave cones. The resultant spectral sensitivities thus are assumed to reflect the middle- and long-wave sensitive cones. There is good evidence that the middle- and long-wave cones are represented in equal ratios in the fovea and parafovea.\textsuperscript{23,25}

The subjects manipulated the radiance of the comparison field by adjusting that channel's neutral-density wedge such that, in counterphase with the standard, a point of minimum flicker was achieved. This was done three times for the fovea of one eye at a comparison wavelength of 460 nm. The subject then repeated this series of measurements at a comparison wavelength of 530 nm. Then three measurements were made at these two comparison wavelengths for the parafovea of the same eye after pupillary realignment and 4 min of parafoveal adaptation to the background field. This entire sequence of events then was repeated for the other eye after a brief rest period. Five such sessions were run for each subject, each session being conducted on a separate day. Retinal locus and eye were counterbalanced across sessions.

**Calibration**

The relative spectral energy of the comparison fields was measured at the end of every experimental session. This was accomplished by placing a calibrated photodetector in the path of the light from each of the two comparison fields while all other sources of light in the system were blocked. These values were used in the analysis of the data to control for daily differences in the relative radiance of the 460- and 530-nm comparison lights.

**Results**

For one subject, HFP was used to measure spectral sensitivity in the fovea and in the parafovea to lights with 450–530-nm wavelength in 10-nm steps. Taking the difference in log spectral sensitivity at these two retinal loci yielded a curve that matched the absorption spectrum for macular pigment. Figure 1 illustrates the difference spectrum, as measured by the described technique. The data were normalized by a scalar to a $\lambda_{\text{max}} = 0.50$ at 460 nm for direct comparison with the macular pigment absorption spectrum obtained by microspectrophotometry on fixed tissue.\textsuperscript{8} There was a suggestion that measurements made on fresh tissue would provide an even better fit.\textsuperscript{8} These two sets of data also were compared with a derived template.\textsuperscript{26} The data obtained by our in vivo psychophysical technique showed close agreement with these in situ measurements\textsuperscript{8} and template.\textsuperscript{26} This agreement verified that the procedures and stimulus conditions used in our experiment were adequate to obtain macular pigment OD measurements reliably.

The OD of macular pigment was derived on the basis of the following equation:

$$\text{DO}_{\text{MP}} = \log S_{\text{F}_{460}} - \log S_{\text{F}_{530}} + \log S_{\text{P}_{350}} - \log S_{\text{P}_{330}}$$

(1)

where, with lights of 460 and 530 nm, $S_{\text{F}}$ is the foveal sensitivity, and $S_{\text{P}}$ is the parafoveal sensitivity. The sensitivity values were based on the median of three wedge settings for a given condition. The mean OD for our ten subjects was 0.32 (range, 0.05–0.66). This agreed generally with earlier results\textsuperscript{14} obtained under similar experimental conditions. The mean OD for the 50 subjects in this other experiment was 0.39 (range, 0.10–0.80).

Exploratory data analysis confirmed that the data for each subject met the assumptions of normality, linearity, and homoscedasticity. A two-sample student t-test was done to assess whether the absolute
Table 1. Macular pigment O.D. for the left and right eyes of ten subjects for each of five sessions, averaged across sessions

<table>
<thead>
<tr>
<th>Subject</th>
<th>Session 1</th>
<th>Session 2</th>
<th>Session 3</th>
<th>Session 4</th>
<th>Session 5</th>
<th>Mean</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>KF</td>
<td>0.34</td>
<td>0.40</td>
<td>0.32</td>
<td>0.34</td>
<td>0.26</td>
<td>0.38</td>
<td>0.30</td>
<td>0.27</td>
</tr>
<tr>
<td>MD</td>
<td>0.50</td>
<td>0.46</td>
<td>0.38</td>
<td>0.52</td>
<td>0.43</td>
<td>0.43</td>
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<td>0.44</td>
</tr>
<tr>
<td>LP</td>
<td>0.05</td>
<td>0.13</td>
<td>0.08</td>
<td>0.07</td>
<td>0.09</td>
<td>0.18</td>
<td>0.08</td>
<td>0.05</td>
</tr>
<tr>
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<td>0.43</td>
<td>0.47</td>
<td>0.43</td>
<td>0.35</td>
<td>0.34</td>
<td>0.41</td>
<td>0.47</td>
</tr>
<tr>
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<td>0.43</td>
<td>0.35</td>
<td>0.37</td>
<td>0.35</td>
<td>0.44</td>
<td>0.27</td>
<td>0.37</td>
</tr>
<tr>
<td>BJ</td>
<td>0.14</td>
<td>0.12</td>
<td>0.25</td>
<td>0.19</td>
<td>0.18</td>
<td>0.05</td>
<td>0.13</td>
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<tr>
<td>JC</td>
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<td>0.66</td>
<td>0.63</td>
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<td>0.64</td>
<td>0.65</td>
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<tr>
<td>BS</td>
<td>0.33</td>
<td>0.34</td>
<td>0.29</td>
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<td>0.29</td>
<td>0.36</td>
<td>0.29</td>
<td>0.34</td>
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<tr>
<td>MK</td>
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<td>0.20</td>
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<td>0.15</td>
<td>0.31</td>
<td>0.29</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Accompanying t values test whether the mean differences between the left and right eyes differ significantly from zero (degrees of freedom = 8).

Differences between the macular pigment density of the left and right eyes were greater than 0. Table 1 presents the macular pigment O.D.s for the left and right eyes of the ten subjects, with the results of the tests of significance.

Significant interocular differences were found in two of the ten subjects. For PG, the right eye was consistently and significantly higher in macular pigment OD than the left eye (t(8) = 3.22, P < 0.01). For BS, the same was true (t(8) = 2.58, P < 0.03). For both subjects, the mean differences were small (PG, 0.08; BS, 0.04). For each of the remaining subjects, there was no consistent pattern of interocular differences. Thus, although some subjects showed interocular effects, in general, the left and right eyes contained the same amount of macular pigment. That this is the case can be seen in the scatterplot in Figure 2.

This figure illustrates the correlation between the overall mean left and right eye macular pigment ODs of the ten subjects. Although this correlation was fitted best by a slope of 0.92 (r = 0.96), a slope of 1.0 was required to conclude that no interocular differences generally existed. The obtained slope did not differ significantly from 1.0 (P < 0.142).* The data points in Figure 2 are scattered around, suggesting that subjects differed from one another in terms of the amount of their macular pigment. Two-way analysis of variance indicated that there were significant individual differences in macular pigment OD when the OD values of the two eyes of each subject were averaged (F(9, 90) = 45.19, P < 0.0001).

Although most subjects showed no overall interocular differences in macular pigment OD, in some individual sessions, the amount of macular pigment in one eye compared with the other seemed to differ considerably, by as much as 72% (e.g., Table 1, BJ, session 3). To conclude that such differences are statistically significant for a given session, it is necessary to obtain a number of density measurements from both eyes. Given the procedure we used, it was possible to derive only a single value for each eye per session. To obtain a large enough sample to provide the appropriate statistical power, the test session would have been excessively long. As an alternative, we relied on a resampling technique described as follows. Because we used three trials (repeated measures) in each condition and four conditions for each eye (the foveal and parafoveal 460-nm and the foveal and parafoveal 530-nm

* This was based on a one-tailed Student's t-test using the distribution of slopes.
ences to imperfect dissections and concluded that no systematic effects were found.

Discussion

Whether interocular differences exist in macular pigment density is controversial. With microdensitometry, it was originally reported that the amount of macular pigment between the two eyes of a single subject was similar. However, with high-performance liquid chromatography, the opposite conclusion was reached. More recent studies, using high-performance liquid chromatography and microdensitometry, reported no interocular differences in zeaxanthin (one of the carotenoids comprising macular pigment), but significant interocular differences in lutein (the other carotenoid in the macular pigment). These authors attributed the lutein differences to imperfect dissections and concluded that no interocular differences actually exist in either zeaxanthin or lutein. All these studies were done with excised eyes, thus allowing for the possible influence of postmortem effects (such as degradation of the retina) or artifacts caused by the intrusion of chemical fixation agents. In our in vivo study, psychophysical measurements of macular pigment density were made, and the results were analyzed statistically.

This analysis of our data showed significant and consistent interocular differences in only two of ten subjects. In these two, the interocular differences were small, especially in comparison with the overall OD differences found between subjects. These latter differences agreed with those in earlier reports. In the remaining eight subjects, we frequently found interocular differences in macular pigment density for a given session. To determine if these differences were statistically significant, the resampling technique described earlier was used, and student t-tests were applied to the generated distributions. Our results showed a number of significant intrasession interocular differences. These differences were as great as 72% (average difference, 19%) but were not systematic (consistently greater in one eye across sessions). Furthermore, although our results were suggestive, they should be treated with caution. Because of the lack of independence inherent in the resampling data, the student t-tests used to estimate the significance of the interocular differences may have been overly sensitive. We also found considerable daily differences in macular pigment density for a given eye. Comparing the highest OD value with the lowest OD value for each of the ten subjects in the right eye only, we obtained across-session differences as high as 75% (average difference, 37%). Given such day-to-day variability, it is therefore possible (perhaps even likely) to find interocular differences on the basis of a single measurement, regardless of the source of the variability, which might include measurement error. This could account for the findings of others that differences as high as 69% can occur (average difference, 29% across the two eyes of 36 subjects). Only one measurement per subject was made in this study. Others found differences only as high as 13% (average, 6%) for zeaxanthin and up to 15% (average, 11%) for lutein. These results were found in a considerably smaller sample (n = 6).

To summarize, we showed that although, interocular differences exist in a limited number of cases and to a small extent, in general, the amount of macular pigment in one eye is essentially the same as in the fellow eye. However, on any given day, even among subjects who do not show consistent interocular differences, interocular differences may exist. In addition, there are day-to-day differences in measurements of macular pigment density even in the same eye. What contributes to this variation has yet to be determined, but diet may be a likely source. The mechanism responsible for the uptake and deposition of ocular carotenoids is poorly understood and may also contribute to this variability. Finally, although statistically significant interocular differences were shown to exist for a small number of subjects in this study, the differences were small, and their biologic significance should be questioned.

Key words: macular pigment, interocular differences, carotenoids, color vision

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References